JOURNAL OF VIROLOGY, Mar. 1983, p. 1195–1199
0022-538X/83/031195-05$02.00/0

Vol. 45, No. 3

Cloning and Analysis of Reverse Transcript P160 Genomes of Abelson Murine Leukemia Virus

SAMUEL A. LATT,† STEPHEN P. GOFF,‡ CLIFFORD J. TABIN, MICHAEL PASKIND, JEAN Y.-J. WANG, AND DAVID BALTIMORE*

Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, and Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02139

Received 27 September 1982/Accepted 22 November 1982

Circular duplex reverse transcripts of the genome of a strain of Abelson murine leukemia virus that encodes a 160,000-molecular-weight protein were isolated, cleaved with *Hin*dIII restriction endonuclease, and cloned into the unique *Hin*dIII site of lambda phage Charon 21A. Recombinant phage clones, some of which were infectious in transfection assays, were found to contain a 789-base-pair region specific for Abelson murine leukemia virus; this region is not found in other strains of this virus. The extra sequence was localized by restriction endonuclease and electron microscopic heteroduplex analysis. Sequence analysis showed no homology at the ends of the extra sequence, implying that it was deleted by an event that did not utilize sequence homology. The sequence of this unique region has an open reading frame through its entirety.

Abelson murine leukemia virus (A-MuLV) is a replication-defective retrovirus capable of transforming mouse lymphoid (1, 13) and fibroblastic (15) cells. The genome of this virus is a hybrid RNA molecule containing portions of the parental Moloney MuLV at its ends and a large substitution, termed v-*abl*, in the center (16). This central substitution is homologous to a cellular gene, termed c-*abl*, which is apparently interrupted by several intervening sequences not present in the viral genome (7).

Several variants of A-MuLV have been isolated with alterations in the central portion of the genome. These variants encode related membrane-associated proteins ranging in size from 160,000 to 85,000 daltons (14). The largest genome, that of A-MuLV(P160), is approximately 6.2 kilobases (kb) long and is known to encode the largest of these polypeptides, termed P160. Other genomes, including those encoding proteins P120, P100, and P90, are smaller and apparently share a common deletion of 0.7 to 0.8 kb with that encoding P160 (7, 9). A transformation-defective genome (21) encoding a protein termed P92*td* has suffered yet another deletion (9).

To isolate DNA clones representing the genome of A-MuLV(P160), virus was isolated from the A-MuLV(P160)-transformed NIH 3T3 fibroblast cell line AN-P160-54 (9), which had been coinfected with the clone 3A strain of Moloney MuLV. The virus was used to infect sensitive NIH 3T3 cells from which supercoiled circular duplex A-MuLV(P160) DNA was isolated (7). This DNA was cleaved with *Hin*dIII endonuclease and cloned into the unique *Hin*dIII site of lambda phage Charon 21A (20). A total of 10 independent isolates that hybridized (4) to an *abl*-specific probe (plasmid pAB3SUB3) (7) were grown into phage stocks, the DNA from which was characterized for restriction endonuclease cleavage sites. Preliminary restriction maps of these 10 clones were prepared by using the *abl*-specific probe to identify (19) major restriction fragments. Five of the clones appeared to contain full-length A-MuLV(P160) genomes with one long terminal repeat (LTR) sequence (6.2 kb total), and three had apparently full-length genomes with two tandem LTR sequences (6.7 kb total). The remaining two clones contained large internal deletions (1.0 and 0.2 kb) and were not characterized further (Table 1).

Comparison of the restriction enzyme cleavage maps of these clones with those of other strains of A-MuLV DNA (9) suggests that the A-MuLV(P160) genome contains an extra 0.7 to 0.8 kb when compared with the P120, P100, or P90 strains and that a new *Sac*I site is present in this extra DNA. This unique sequence was localized near the center of the *abl* region. In addition, the LTR region of the P160 clones was

† Present address: Genetics Division, Childrens Hospital Medical Center, Boston, MA 02115.
‡ Present address: Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, NY 10027.

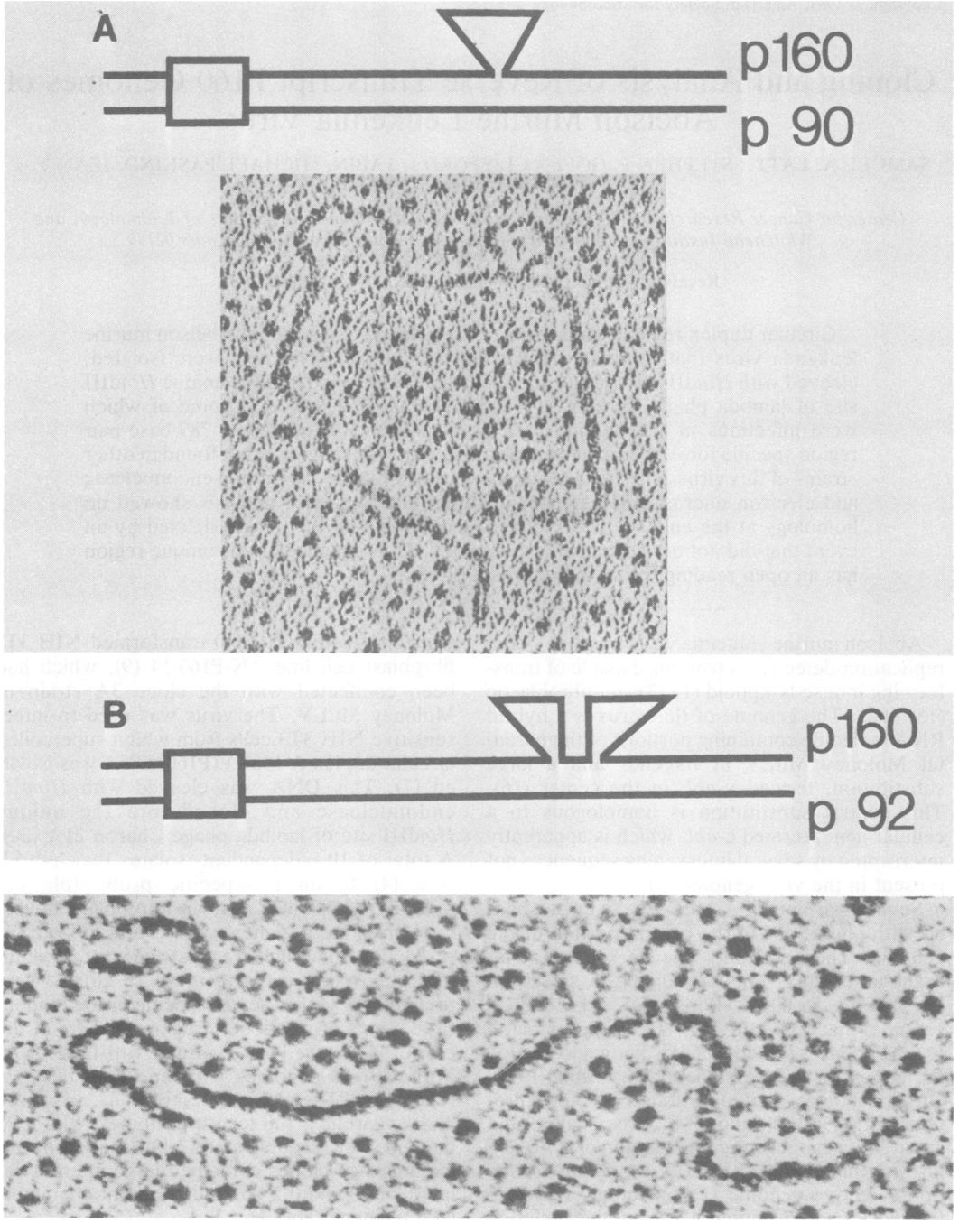

FIG. 1. (A) Heteroduplex (diagrammed in inset) between cloned inserts from A-MuLV(P160) and A-MuLV(P90). (B) Heteroduplex (diagrammed in inset) between cloned inserts from A-MuLV(P160) and A-MuLV(P92*td*).

approximately 80 base pairs (bp) shorter than that of the other A-MuLV genomes examined.

A prototype clone with one LTR (λAB160-1) was chosen for further analysis. Examination of heteroduplexes (6) between λAB160-1 and λAB3 [derived from A-MuLV(P90)] (7), revealed complete homology, except for a single loop, 0.73 kb in size, mapping 1.7 kb from one end of the

TABLE 1. Properties of A-MuLV(P160) clones

Clone	No. of LTRs	Transformation[a]
λAB160-1	1	−
λAB160-2	1	+
λAB160-3	1	+
λAB160-5	2	+
λAB160-6	1	−
λAB160-9[b]	1	−
λAB160-12	2	+[d]
λAB160-13	2	−
λAB160-15	1	+[d]
λAB160-16[c]	1	−

[a] Each DNA preparation was cleaved with *Hin*dIII endonuclease and religated to produce some DNA fragments containing a nonpermuted copy of the provirus. These DNA samples were then cotransfected as described in the text with a 10-fold molar excess of DNA of a biologically active proviral clone of Moloney MuLV termed pZAP (18). The cells were replated 1 day later, and foci were scored after 15 to 20 days in culture.

[b] Deletion of 1.0 kb in 2.75-kb *Sac*I-*Sac*I fragment (Fig. 3).

[c] Deletion of 0.2 kb in 2.75-kb *Sac*I-*Sac*I fragment (Fig. 3).

[d] Transformed cells produced P160.

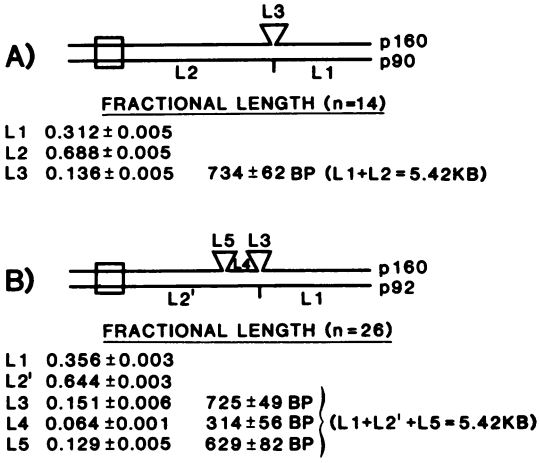

FIG. 2. Analysis of heteroduplex data involving cloned A-MuLV molecules. Heteroduplex figures were identified on 8 by 10 in (20 by 25 cm) photographic prints. Relative segment lengths were determined from duplicate scans with an X-Y digitizer kindly made available by Jonathan King. (A) A-MuLV(P160)–A-MuLV(P90) heteroduplex. (B) A-MuLV(P160)–A-MuLV(P92*td*) heteroduplex. The segments identified in the diagram based on the number of pictures (n) indicated in parentheses are represented first as average fractional lengths (± standard error of the mean), from which the sizes of the inserted segments are estimated.

inserts (Fig. 1A). Thus, the extra DNA contained in the A-MuLV(P160) genome apparently lies in a single contiguous stretch. Heteroduplexes between λAB160-1 and λAB2 [derived from A-MuLV(P92*td*)] (7) showed two separate deletion loops 0.7 and 0.6 kb in size, separated by 0.3 kb of duplex DNA (Fig. 1B). These data (summarized in Fig. 2) show that the two deletions present in A-MuLV(P92*td*) do not overlap.

To determine the sequence of the unique information in A-MuLV(P160), a 0.94-kb *Pst*I fragment of λAB160-3, which appeared to contain the entire unique region, was subcloned into the *Pst*I site of plasmid pBR322 (5). Comparison of the sequence (12) of this fragment with that of an A-MuLV(P90) clone (Lee et al., manuscript in preparation) shows that the unique region is 789 bp long and has a single open reading frame through its entirety. The sequence itself will be published as part of the complete A-MuLV sequence (manuscript in preparation). Certain restriction enzyme cleavage sites are shown in Fig. 3. DNA sequence and restriction fragment analysis placed the 3′ boundary of the A-MuLV(P160)-specific insert 1.75 kb interior to the 3′ end of the total A-MuLV(P160) genome, as compared with 1.69 ± 0.05 kb estimated by heteroduplex analysis. Agreement between these two estimates, and between the insert size as determined by DNA sequencing (789 bp) and by electron microscopy (725 ± 48 and 734 ± 62 bp) (Fig. 2), is reasonably good.

An important aspect of the unique sequence in λAB160-3 is that there is no homology between the end points of the region (Fig. 4), implying that if (as seems most likely) A-MuLV(P160) suffers a deletion to produce the A-MuLV(P120) genome, that deletion involves no homology. In addition, the sequence at the deletion is not compatible with the idea that an aberrant splic-

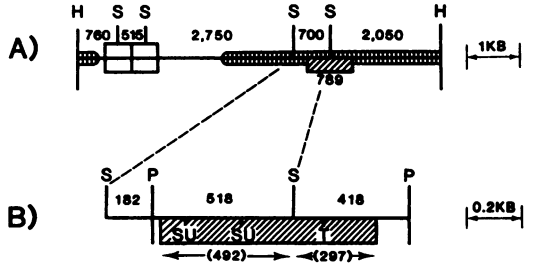

FIG. 3. Summary map of A-MuLV(P160) genomes derived from electrophoresis and heteroduplex data. (A) Insert with two LTRS. (B) Detail of (A). ▭, v-*abl* sequences. Scale is indicated at the right. Enzyme cleavage sites are indicated as follows: H, *Hin*dIII; S, *Sac*I; P, *Pst*I; T, *Taq*I; SU, *Sau*96I. The size of the A-MuLV(P160)-specific insert (▭) is based on DNA sequence data. The data in (B) are derived largely from analysis of the subcloned *Pst*I fragment.

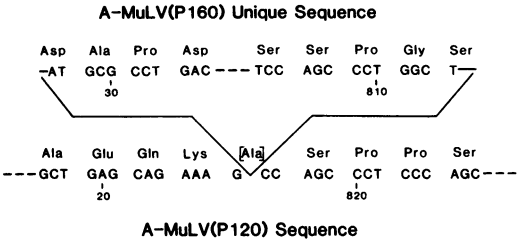

FIG. 4. DNA sequences at the junctions of the A-MuLV(P160)-specific DNA insert. Base pairs are numbered starting with the 5′ terminus of the *Pst*I fragment. The boundaries of the surrounding A-MuLV(P120) sequence are also indicated. Written above each triplet is the corresponding amino acid, and in the case of the A-MuLV(P120) sequence, the amino acid (in brackets) replaced in A-MuLV(P160) because the insert occurred within a codon.

ing event causes the removal of the 789 bp; no homology to the consensus sequences at splice joints (2) was detected. One mechanism that could generate the deletion is an unequal, illegitimate recombination between A-MuLV(P160) genomes, such as that postulated by Goldfarb and Weinberg (10) to occur between deleted Harvey sarcoma virus and Moloney MuLV genomes.

The cloned permuted A-MuLV(P160) genomes were tested for biological activity in a transfection assay (8) as modified by Andersson et al. (3) from the method of Graham and van der Eb (11), with NIH 3T3 cells used as indicator cells. Of eight independent clones lacking a deletion in the *abl* sequence, five were capable of transforming mouse NIH 3T3 cells (Table 1). Preliminary data (Rotter, unpublished) are consistent with the production of the P160 protein by most, if not all, of these transfectants. Three of the infective clones contained one LTR; the other two contained two LTRs. The LTRs of these A-MuLV(P160) clones were shorter by approximately 80 bp than the LTRs of other A-MuLV strains. The exact position of this deletion has not yet been determined, but it might well correspond to loss of one of the tandemly repeated 75-bp sequences starting 117 and 192 bp from the 5′ end of the LTR in M-MuLV (17). The biological significance of this deletion is not yet clear. Since it was present in all 10 of the A-MuLV(P160) clones examined, it presumably preexisted in the A-MuLV(P160) RNA and hence was compatible with biological transformation.

The acquisition and initial characterization of A-MuLV(P160) clones should permit more detailed study of the *abl* sequence segments in the mouse genome and of structure-activity relationships within the family of transformation-associated A-MuLV proteins.

We thank Chris Riser for advice on the use of the electron microscope.

This work was supported by Public Health Service grants CA-26717 (to D.B.), GM-21121 (to S.A.L.), and CA-14501 (core grant to S. E. Luria) from the National Institutes of Health. S.P.G. was a postdoctoral fellow of the Jane Coffin Childs Memorial Fund for Medical Research, as is J.Y.-J.W. D.B. is an American Cancer Society Research Professor.

LITERATURE CITED

1. **Abelson, H. T., and L. S. Rabstein.** 1970. Lymphosarcoma: virus-induced thymic-independent disease in mice. Cancer Res. **30**:2213–2222.
2. **Abelson, J.** 1979. RNA processing and the intervening sequence problem. Annu. Rev. Biochem. **48**:1035–1069.
3. **Andersson, P., M. P. Goldfarb, and R. A. Weinberg.** 1979. A defined subgenomic fragment of in vitro synthesized Moloney sarcoma virus DNA that can induce cell transformation upon transfection. Cell **16**:63–75.
4. **Benton, W. D., and R. W. Davis.** 1977. Screening gt recombinant clones by in situ hybridization to single plaques. Science **196**:180–181.
5. **Bolivar, F., R. L. Rodriquez, P. J. Green, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–111.
6. **Ferguson, J., and R. W. Davis.** 1978. Quantitative electron microscopy of nucleic acids, p. 123–171. *In* J. K. Koehler (ed.), Advanced techniques in biological electron microscopy. Springer-Verlag KG, Berlin.
7. **Goff, S. P., E. Gilboa, O. N. Witte, and D. Baltimore.** 1980. Structure of the Abelson murine leukemia virus genome and the homologous cellular gene: studies with cloned viral DNA. Cell **22**:777–785.
8. **Goff, S. P., C. J. Tabin, J. Y.-J. Wang, R. A. Weinberg, and D. Baltimore.** 1982. Transformation of fibroblasts by cloned Abelson murine leukemia virus DNA and recovery of transmissible virus by recombination with helper virus. J. Virol. **41**:271–285.
9. **Goff, S. P., O. N. Witte, E. Gilboa, N. Rosenberg, and D. Baltimore.** 1981. Genome structure of Abelson murine leukemia virus variants: proviruses in fibroblasts and lymphoid cells. J. Virol. **38**:460–468.
10. **Goldfarb, M. P., and R. A. Weinberg.** 1981. Generation of novel, biologically active Harvey sarcoma viruses via apparent illegitimate recombination. J. Virol. **38**:136–150.
11. **Graham, F. L., and A. J. van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus DNA. Virology **52**:456–471.
12. **Maxam, A. M., and W. Gilbert.** 1977. A new method for sequencing DNA. Proc. Natl. Acad. Sci. U.S.A. **74**:560–564.
13. **Rosenberg, N., and D. Baltimore.** 1980. Abelson virus, p. 7187–7203. *In* G. Klein (ed.), Viral oncology. Raven Press, New York.
14. **Rosenberg, N., and O. N. Witte.** 1980. Abelson murine leukemia virus mutants with alterations in the virus-specific P120 molecules. J. Virol. **33**:340–348.
15. **Scher, C. D., and R. Siegler.** 1975. Direct transformation of 3T3 cells by Abelson murine leukemia virus. Nature (London) **253**:729–731.
16. **Shields, A., S. P. Goff, M. Paskind, G. Otto, and D. Baltimore.** 1979. Structure of the Abelson murine leukemia virus genome. Cell **18**:955–962.
17. **Shinnick, T. M., R. A. Lerner, and J. G. Sutcliff.** 1981. Nucleotide sequence of Moloney murine leukaemia virus. Nature (London) **293**:543–548.
18. **Shoemaker, C., J. Hoffmann, S. P. Goff, and D. Baltimore.** 1981. Intramolecular intergration within Moloney leukemia virus DNA. J. Virol. **40**:164–172.

19. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
20. **Williams, B. G., and F. R. Blattner.** 1980. Bacteriophage lambda vectors for DNA cloning, p. 201–281. *In* J. Setlow and A. Hollaender (ed.), Genetic engineering: principles and methods, vol. 2. Plenum Publishing Corp., New York.
21. **Witte, O. N., S. P. Goff, N. Rosenberg, and D. Baltimore.** 1980. A transformation-defective mutant of Abelson murine leukemia virus lacks protein kinase activity. Proc. Natl. Acad. Sci. U.S.A. **77**:4993–4997.